Metabolic rhythms of unicellular, nitrogen fixing cyanobacteria and possible interplay with modeled KaiABC circadian oscillator

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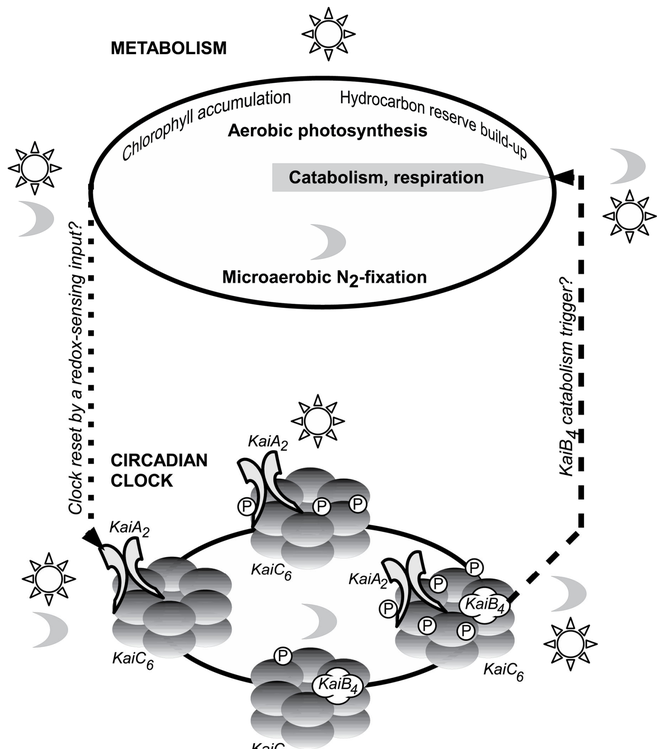
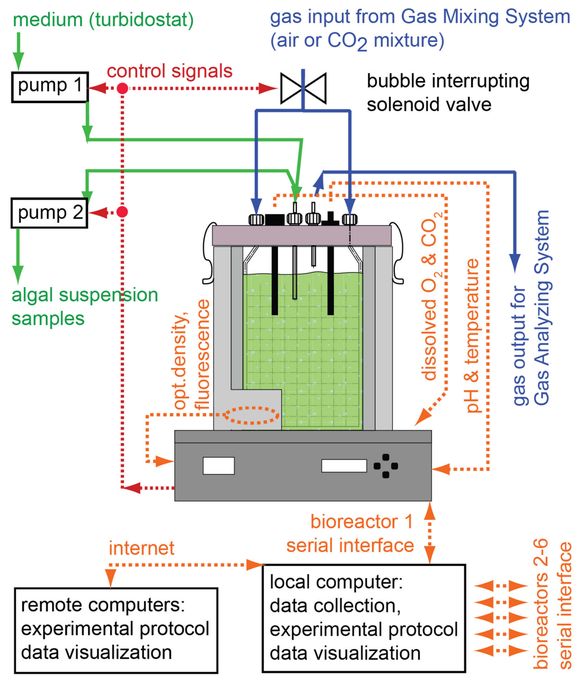
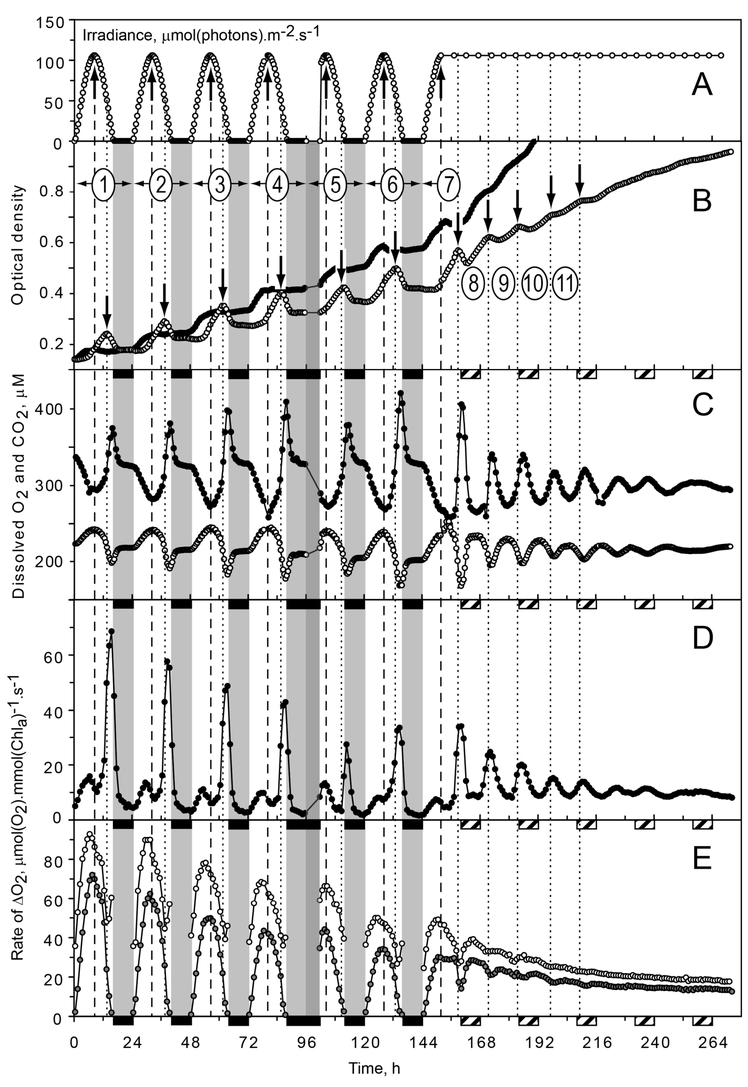
Abstract

Circadian clocks of living organisms provide evolutionary advantage and make living in dynamic environment more efficient. The clocks affect human lives and health as well as control the simplest organisms such as prokaryotic cyanobacteria. The cyanobacteria represent an excellent model that is amendable to a multitude of genetic, biochemical, and biophysical methods. The cyanobacterium, *Cyanothece* sp. ATCC 51142 relies on the circadian clock to permit, in the same cell, anoxygenic nitrogen fixation at night and oxygenic photosynthesis during solar day. We measured real-time, in-situ photosynthetic and respiratory activities as well as the culture growth under light forcing conditions and also under constant light, i.e. free-running mode. Interestingly, the experiments show a strong 24h-period dynamic pattern that is replaced by apparent 12h-period in free running mode. The 24h-pattern does not change significantly when changing the light/dark ratio from 16hL/8hD to 12hL/12hD and 8hL/16hD. Furthermore, we tried to elucidate connection between these metabolic rhythms and known structure of KaiABC circadian oscillator by means of mathematical modeling. One of simulation results show a strong correlation between the presumed catabolic event indicated by significant peak in respiratory activity, and simulated dynamics of $KaiB_4$ complex in modeled circadian pacemaker. A causal relationship between these 2 events is suggested because $KaiB_4$ facilitates dephosphorylation of KaiC6 hexamer, which is known to signal upcoming dark period.

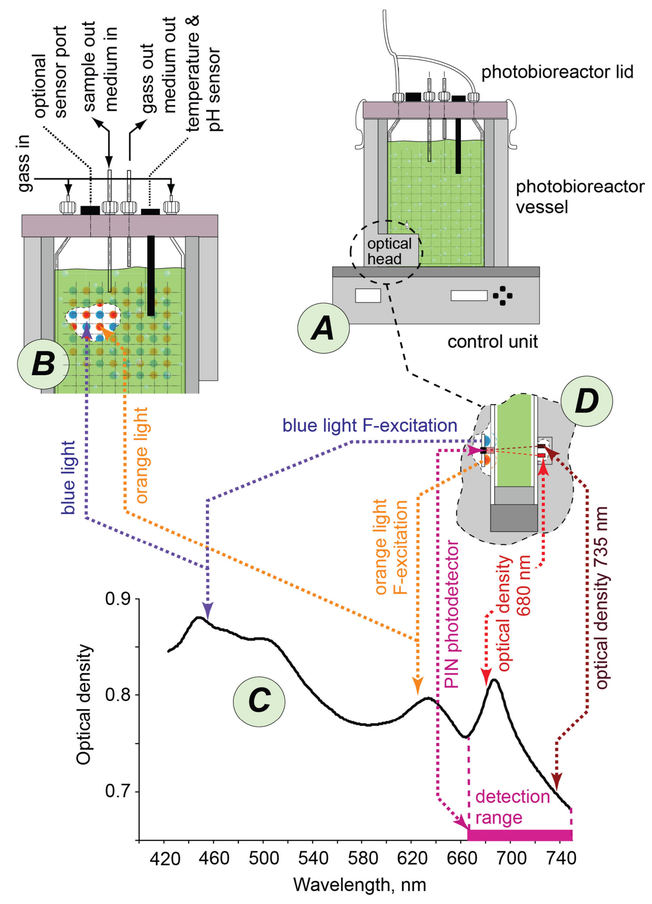


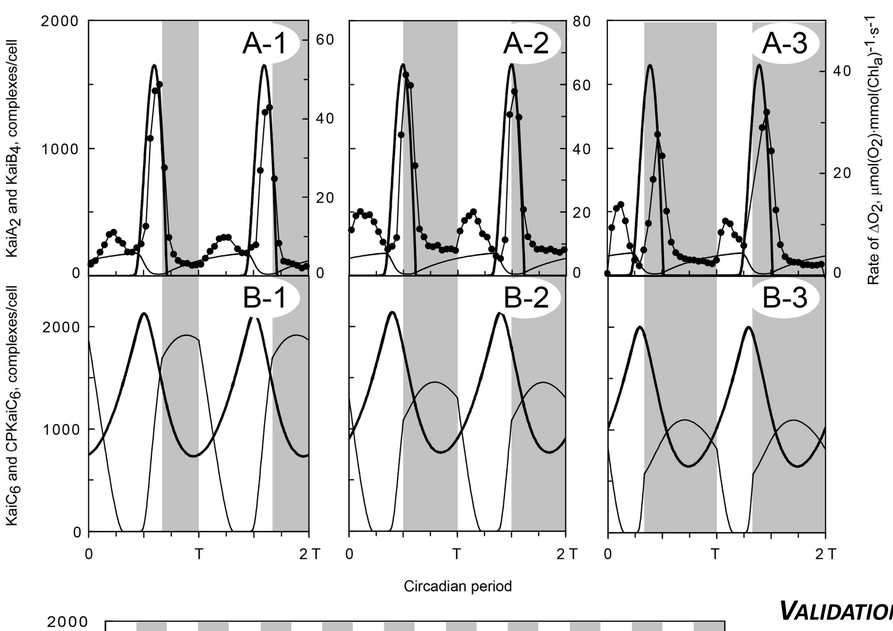




Experiment

Figure represents the dynamics of the *Cyanothece* culture in two regimes. First, the culture was forced by six 24 hour cycles of 16 hour light periods alternating with 8 hours of darkness (grey vertical columns). In the second part of the experiment at noon of the 7th day the diurnal light modulation was replaced by constant irradiance to investigate the free-running mode of the cyanobacterial dynamics. Panel *A* shows photosynthetically active photon flux density in 24 hour periods, numbered *1-6*. The vertical dashed lines indicate noon with maximum irradiance. The vertical grey bars together with the black horizontal bars on the time axis indicate 8 h long periods of darkness. Panel *B* shows optical density of the *Cyanothece* sp. suspension at 735 nm (OD_{735}, open circles) and the difference OD_{680}-OD_{735} correlate with biomass and chlorophyll concentration, respectively (Nedbal et al., 2008). The vertical dotted lines and down pointing arrows show local maxima of OD_{735}. Panel *C* shows concentration of O_2 (open circles) and of CO_2 (closed circles) dissolved in the cyanobacterial suspension. Panel *D* then shows rate of oxygen consumption measured during 3 minutes of darkness (Červený et al. 2009). Finally panel *E* shows rate of oxygen evolution at the bioreactor irradiance level at the time of the measurement (grey filled circles), and at saturating irradiance of 260 µmol(photons).$m^{-2}.s^{-1}$ (open circles). In contrast to respiration, the photosynthetic oxygen evolution rate follows changes in incident irradiance very closely. In the free-running mode, with an exception of the rate dip at around 157th hour of the experiment that comes in an anticipation of upcoming night, the oxygen evolution rate declines steadily. The oxygen evolution rate at the saturating irradiance exhibits a local minimum followed by a transient rise just at the time of the respiratory peak. This feature, however, fades away rapidly in the free-running mode.





Mathematical models

The dynamic pattern observed experimentally in the cyanobacterial metabolism is confronted with the circadian clock model prediction. Simulated dynamics of the $KaiA_2$ (*A* panels, thin lines) and $KaiB_4$ complexes (*A* panels, heavy lines) and measured respiratory O_2 uptake (*A* panels, closed circles) in the LD 16:8 (*A-1*), LD 12:12 (*A-2*), and LD 8:16 regimes (*A-3*) are compared. The dynamics of the non-phosphorylated $KaiC_6$ (*B*, thin lines) and the fully phosphorylated $CPKaiC_6$ (*B*, heavy lines) hexamer complexes are shown in the *B* row of panels.

The simulation results show a strong correlation between the presumed catabolic event marked by the peak in respiratory activity, and the simulated dynamics of the $KaiB_4$ complex in the circadian pacemaker. A causal relationship between these two events is suggested because $KaiB_4$ facilitates de-phosphorylation of $CPKaiC_6$ (e.g., Dong and Golden, 2008; Johnson et al., 2008), which is known to signal the upcoming night of the circadian clock. Our conclusions rely, in part, on use of the circadian clock model by Miyoshi et al. (2007) that was derived for *Synechococcus elongatus* rather than for *Cyanothece* sp. The dynamics of the CO_2 distribution in algal photobioreactor influenced by organism metabolic activity can be further modeled as described in our recent paper (Nedbal et. al. 2010).

Validation of the model

The validity of the model was challenged in a simulation of KaiABC dynamics during half-circadian (T=~12 h) forcing of LD 6:6 (upper figure). The model predicted irregular occurrence of $KaiB_4$ accumulation events rather than damping (panel A, heavy line). In correlating $KaiB_4$ level with respiratory peaks, one might expect that during experiments with the LD 6:6 regime both would show a similar random pattern. Indeed, we found a qualitative agreement between the simulated and measured irregular dynamic patterns of $KaiB_4$ (top figure) and of respiratory O_2 uptake (bottom figure), respectively, and we interpret this agreement as a validation of the Miyoshi model.

We propose a hypothesis that respiration is controlled by the circadian clock *via* a direct and nearly instantaneous interaction with the active protein tetramer $KaiB_4$ (or with a *Cyanothece* $KaiB_4$ analogue). The hypothesis is based on the robust correspondence between the experimentally observed respiratory peak and the modeled $KaiB_4$ abundance. This model-based hypothesis must be verified by linking the respiratory peak to the experimentally measured concentration of the active $KaiB_4$ tetramer.

Aperiodic oscillations in the form of chaos may occur if the amplitude of forcing LD cycles becomes sufficiently large as proposed by theoretical study (Gonze and Goldbeter, 2000).

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